

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Research paper

Synthesis and pharmacological evaluation of some new fluorine containing hydroxypyrazolines as potential anticancer and antioxidant agents





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ARTICLE INFO

Article history: Received 17 April 2015 Received in revised form 14 September 2015 Accepted 23 September 2015 Available online 28 September 2015

Keywords: Synthesis Hydroxypyrazolines Characterization Anticancer activity Antioxidant activity

1. Introduction

ABSTRACT

Breast cancer is probably the most prevalent cancer in women. The development of resistance to therapeutic agents and lack of targeted therapy for breast cancer cells provide motivation to identify new compounds for the treatment. With this objective in mind, a new series of 3-fluoro-4-methoxyphenyl group based 1,3,5-trisubstituted aryl-5-hydroxypyrazoline analogues **4a**–**1** was synthesized through multi-step reaction sequence. The structures of the newly synthesized compounds were confirmed by IR, ¹H NMR, ¹³C NMR, LC-MS and elemental analysis. They were screened for their *in vitro* anticancer and *in vitro* antioxidant activities. Among the tested compounds **4h**, **4c** and particularly **4i** displayed promising cytotoxic effect on breast cancer cell lines. The compounds were also found to possess antioxidant activity when tested against DPPH free radical. Overall, this work has contributed to the development of promising leads for anticancer and antioxidant activities.

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Cancer, the uncontrolled, rapid and pathological proliferation of abnormal cells, is among the major worldwide health problems. Despite significant advances in diagnostic and therapeutic techniques, it is now the second major fatal ailment liable for 8.2 million deaths in 2012 [1]. Breast cancer is one of the most commonly diagnosed cancers, accounting for ~20% of all malignancies worldwide and over half a million women develop breast cancer every year. In India, almost 100,000 women are diagnosed with breast cancer every year and a rise to 131,000 cases is predicted by 2020 [2,3]. In general, breast cancer is broadly classified as an endocrine receptor (i.e., estrogen receptor or progesterone receptor), positive or negative. Numerous studies have revealed that estrogens are predominantly active in the initiation and proliferation of breast cancer. Many women ultimately develop metastatic breast carcinoma, which is essentially an incurable disease and the

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http://dx.doi.org/10.1016/j.ejmech.2015.09.029 0223-5234/© 2015 Elsevier Masson SAS. All rights reserved. prognosis has changed little over the past decade [4]. Among all the current therapeutic methods, chemotherapy has become one of the most significant treatment modalities in cancer management. Many current breast cancer chemotherapy treatments are often associated with side effects and the development of drug resistance in cancer cells, whereby majority of the patients succumb to their disease within 2 years of diagnosis. Hence, there is a great need for novel small molecules with the potential to effectively manage the different breast cancer subtypes [5-7].

In the last few decades, research has been focussed on chemically synthesized or natural product derived compounds as anticancer entities. Heterocyclic ring systems have emerged as powerful scaffolds for many biological evaluations. Heterocyclic compounds provide scaffolds on which pharmacophores can arrange to yield potent and selective drugs [8]. Triaryl substituted heterocyclic class of compounds represented by structure **A** (Fig. 1) have attracted considerable interest in the development of potential anticancer agents. Pyrazole and pyrazoline are prominent structural motifs found in numerous antitumor agents [9]. Several 1,3,5-triaryl-4-alkyl-pyrazoles have been evaluated as breast cancer treatments with the goal of reducing both toxicity and increasing



Fig. 1. Structural relatedness of hydroxypyrazolines (C) and triaryl substituted pyrazole (B) analogues with the triaryl substituted heterocyclic model (A).

the response rate as an antitumor agent [10]. Ying Huang and John Katzenellebogen found that triaryl-substituted pyrazole represented by structure **B** (Fig. 1), was good ligand for ER [11]. Since the development of triaryl substituted heterocyclic template has formed the backbone of a multitude of non-steroidal estrogen agonists as well as antagonists.

Numerous studies have demonstrated that in addition to cancer, oxidative stress on the cell has increased dramatically. The oxidative stress reflects an imbalance between the oxidants and the antioxidant favoring the oxidants implies damage of all essential bio-compounds like proteins, DNA and membrane lipids and can result in cell death [12]. There is increasing evidence showing the involvement of oxidative stress induced by free radicals and reactive oxygen species (ROS) in a variety of diseases and pathophysiological events including inflammation, cancer, myocardial infraction, arthritis and neurodegenerative disorders [13]. Antioxidants can minimize or inhibit the oxidative damage by interrupting the free radical formation or terminating the chain reaction. Antioxidants may slow or possibly prevent the development of the above mentioned diseases [14,15]. On the other hand, many chemotherapeutic agents act by producing free radicals, causing oxidative stress in normal cells [16]. A mono therapy of an anticancer drug with antioxidant properties will probably become more advantageous from the pharmaco-economic point of view.

Among the nitrogen heterocycles, pyrazoline and their derivatives are acknowledged to possess a wide range of bioactivities [17]. Pyrazolines are used as anticancer [18,19], antioxidant [20], antitumor [21], anti-inflammatory [22], trypanocidal [23], MAO-B inhibitors [24], agonist of cannabinoid receptors [25] and antidepressant agents [26]. Therefore, the pyrazoline motif makes up the core structure of numerous biologically active compounds. Many fluorinated organic molecules often exhibit remarkable physical and biological properties which originate from the C–F bond with a wide range of applications [27]. Around 20% of all licensed pharmaceutical products over the last 50 years contain a fluorine atom. In the recent past, it was found that fluorinated drugs are metabolically non-degradable, thereby, enhancing the rate of absorption and transfer of the drug to the active site in the body [28]. Since there are very few naturally occurring fluorine-containing compounds and there being a great demand for fluorinated chemicals worldwide, it is necessary to synthesize fluorinated organic compounds. Recently Sharma et al., reported synthesis and in vitro antitumor activity of novel fluorine containing pyrazoles and pyrazolines. These compounds exhibited excellent cytotoxicity against MCF-7 breast cancer cell line [29]. Sarojini and co-workers revealed that fluorine containing hydroxypyrazolines showed potential antiproliferative activity [30]. Based on these aspects, it was decided to report the synthesis and study of in vitro anticancer and in vitro antioxidant activities of a new series of 1,3,5-trisubstituted aryl-5-hydroxypyrazoline analogues.

2. Chemistry

The hydroxypyrazolines **4a**–**I** were synthesized according to the literature method [30] as shown in Scheme 1. In the first step, synthesis of chalcones was carried out by the well-known Claisen–Schmidt reaction, and the products were purified by recrystallization from ethanol (60–70% yield). Chalcones **1a-I** were converted to chalcone dibromides **2a-I**. The intermediate 3-fluoro-4-methoxybenzohydrazide **3** was prepared according to the literature procedure [31]. Condensation of chalcone dibromides **2a-I** with 3-fluoro-4-methoxybenzohydrazide **3** in the presence of triethylamine using absolute ethanol as solvent of reaction gave the desired product. Further purification was done by recrystallization from ethanol.

The structures of the hydroxypyrazolines 4a-l were determined by elemental analysis, IR, ¹H NMR, ¹³C NMR and LC-MS spectral studies (Table 1). In the IR spectrum of hydroxypyrazoline **4c**, a broad absorption band around 3373 cm^{-1} indicated the presence of the hydrogen bonded hydroxyl group in the compound. The amide carbonyl stretching frequency was observed at 1614 cm⁻¹. The shift in the frequency of lower values could be explained on the based on the mesomeric shift and intramolecular hydrogen bonding. The other prominent absorption bands observed in the IR spectrum are 3097 (Ar C-H), 2935 (C–H), 1566 (C=C) and 1176 (C–F) cm⁻¹. The ¹H NMR spectrum of **4c** showed that the proton of the hydroxyl group resonated as a singlet at δ 5.44. The methylene protons of hydroxypyrazoline ring appeared as two doublets centred at δ 3.57 and δ 3.66 with a geminal coupling constant (J = 18 Hz). The appearance of the two doublets clearly reveals the magnetic non-equivalence of the two protons of the CH₂ group adjacent to a chiral centre. The two sharp singlets at δ 3.86 and δ 3.96 assigned for methoxy protons. The four protons of *p*-anisyl moiety resonated as two doublets at δ 6.97 and δ 7.69 (J = 8.8 Hz). Two doublets and one doubledoublet at δ 7.02 (J = 8.4 Hz), δ 7.39 (J = 2 Hz), and δ 7.37 (J = 2.4 Hz) were due to the aromatic protons of the 2,4dichlorophenyl moiety. The protons of 3-fluoro-4methoxyphenyl ring resonated as complex multiplets at δ 7.85–7.94. Further, the ¹³C NMR spectra of **4c** confirmed the presence of a pyrazoline ring by exhibiting signals at δ 48.9 and δ 91.1 as a singlet attached to sp^3 carbons due to C-4 and C-5 carbons, respectively. The two signals due to O-CH₃ carbons appear at δ 55.8 and δ 56.6, and a signal due to the carbonyl carbon appears at δ 162.8. Other aromatic carbons were observed in the expected regions. The LC-mass spectral data of compound 4c provided further evidence of its correct structure. The molecular ion peak was observed at 487 as expected for m/z value $[M-H]^{-}$. Elemental analysis of C, H, and N are within $\pm 0.1\%$ of the predicted values. In the same way, the structures of all the final compounds were confirmed by their characterization data.



R = 2,4-Cl₂, 4-CH₃ **R**₁ = H, 4-CH₃, 4-OCH₃, 4-F, 3-Cl-2-F, 3-F-4-CH₃

Scheme 1. Synthesis of 1,3,5-trisubstituted aryl-5-hydroxypyrazolines (4a-l).

Table 1Characterization data of hydroxypyrazolines 4a–l.

Compound	R	R ₁	Mol. formula	m.p. (°C)	Yield%	Analysis% Found (calculated)		
						С	Н	Ν
4a	2,4-Cl ₂	Н	C23H17Cl2FN2O3	170-172	72	60.12 (60.15)	3.75 (3.73)	6.07 (6.10)
4b	2,4-Cl ₂	4-CH ₃	C24H19Cl2FN2O3	143-145	80	60.88 (60.90)	4.08 (4.05)	5.89 (5.92)
4c	2,4-Cl ₂	4-0CH ₃	C24H19Cl2FN2O4	148-150	76	58.94 (58.91)	3.93 (3.91)	5.68 (5.72)
4d	2,4-Cl ₂	4-F	C23H16Cl2F2N2O3	160-162	65	57.86 (57.88)	3.41 (3.38)	5.85 (5.87)
4e	2,4-Cl ₂	3-Cl-2-F	C23H15Cl3F2N2O3	110-112	68	54.00 (53.98)	2.97 (2.95)	5.50 (5.47)
4f	2,4-Cl ₂	3-F-4-CH ₃	C24H18Cl2F2N2O3	154-156	73	58.65 (58.67)	3.72 (3.69)	5.66 (5.70)
4g	$4-CH_3$	Н	C24H21FN2O3	100-102	71	71.30 (71.27)	5.21 (5.23)	6.96 (6.93)
4h	4-CH ₃	4-CH ₃	C ₂₅ H ₂₃ FN ₂ O ₃	138-140	84	71.73 (71.76)	5.56 (5.54)	6.67 (6.69)
4i	4-CH ₃	4-0CH ₃	C25H23FN2O4	88-90	85	69.09 (69.11)	5.31 (5.34)	6.49 (6.45)
4j	4-CH ₃	4-F	C24H20F2N2O3	231-233	74	68.28 (68.24)	4.79 (4.77)	6.60 (6.63)
4k	4-CH ₃	3-Cl-2-F	C24H19ClF2N2O3	250-252	69	63.12 (63.09)	4.22 (4.19)	6.15 (6.13)
41	4-CH ₃	3-F-4-CH ₃	$C_{25}H_{22}F_2N_2O_3$	163-165	70	68.76 (68.80)	5.10 (5.08)	6.38 (6.42)

3. Pharmacology

3.1. In vitro cytotoxicity assay

Cytotoxicity of all the synthesized compounds were determined on the basis of measurement of *in vitro* growth inhibition of tumor cell lines in 96 well plates by cell-mediated reduction of tetrazolium salt to water insoluble formazan crystals using doxorubicin as a standard. The cytotoxicity as assessed against human breast adenocarcinoma cell lines (MCF-7, MDA-MB-231) and VERO (normal monkey kidney epithelial cell line) using the MTT (3-(4,5dimethyl-2-thiazolyl)-2,5-diphenyl-2*H*-tetrazolium bromide) colorimetric assay [32,33]. The IC₅₀ values (50% inhibitory concentration) were calculated from the plotted absorbance data for the dose–response curves. IC₅₀ values (in μ M) are shown as mean \pm SD of three independent experiments.

3.2. In vitro antioxidant activity using DPPH radical scavenging assay

The hydrogen atom or electron donation ability of the compounds was measured from the bleaching of the purple colored methanol solution of 1,1-diphenyl-1-picrylhydrazyl (DPPH) [34–36]. The spectrophotometric assay uses the stable radical DPPH as a reagent. 1 mL of various concentrations of the test compounds (25, 50 and 100 μ g/mL) in methanol was added to 4 mL of 0.004% (*w*/*v*) methanol solution of DPPH. After a 30 min incubation period at room temperature, the absorbance was read against blank at 517 nm. The DPPH free radical scavenging activity of the molecules, as measured to the conventional antioxidant and was expressed as percent inhibition according to the following formula:

% Inhibition = $[(Abs_{Control} - Abs_{Sample})/Abs_{Blank})] \times 100$

Where, $Abs_{control}$ is the absorbance of the control reaction (containing all reagents, except the test compound) and Abs_{sample} is the absorbance of the test compound. Tests were carried at in triplicate.

4. Results and discussion

4.1. Single-crystal structural characterization by X-ray

X-ray crystallographic data for the compounds were collected at temperature 296 K on Bruker X8 Proteum2 X-ray diffractometer with X-ray generator operating at 45 kV and 10 mA by using CuK_α radiation of wavelength $\lambda = 1.54178$ Å. Data were collected with different settings of φ equal to 0° and 90° by keeping the scan width of 0.5°, exposure time of 5 s and the sample to detector distance, 45.10 mm. A complete data set was processed using *SAINT PLUS*

[37]. The structure was solved by direct methods and refined by full-matrix least squares method on F^2 using *SHELXS* and *SHELXL* [38]. After several cycles of refinement, the final difference Fourier map showed peaks of no chemical significance and the residual is saturated to 0.054. The geometrical calculations were carried out using *PLATON* [39]. The molecular and packing diagrams were generated using *MERCURY* [40].

The spatial structures of compounds **4b** and **4c** were determined by using X-ray diffraction analysis. The single crystals were grown from ethanol solution at room temperature. The molecular views of 4b and 4c are shown in Fig. 2. The crystal and instrumental parameters used in the unit cell determination, the data collection, and structure refinement parameters are presented in Table 2. The study of torsion angles asymmetric parameters and least-squares calculations of compound **4b** reveals that the pyrazoline rings adopts an envelope conformation on C2 and its mean plane makes dihedral angles of $14.76(16)^{\circ}$, $10.70(17)^{\circ}$ and $80.49(17)^{\circ}$ with the fluoromethoxyphenyl, methylphenyl and the dichlorophenyl rings respectively. In the compound 4c, the central pyrazoline ring adopts a twisted conformation on the C3-C2 bond and its mean plane make dihedral angles of 19.38(15)°, 14.55(16)° and 82.07(16)° with the fluoromethoxyphenyl, methoxyphenyl and the dichlorophenyl rings respectively. Both the structures possess a chiral center at C2 with R and S conformations respectively. Since the molecules have crystallized in a centrosymmetric space group, we can surmise that the compounds crystallize as a racemate. The supplementary crystallographic data can be obtained free of charge at www.ccdc.cam.ac.uk/conts/retrieving.html [or from the Cambridge Crystallographic Data Centre (CCDC), 12 Union Road, Cambridge CB2 1EZ, UK; fax: +44(0) 1223 336 033; email: deposit@ ccdc.cam.ac.uk].

4.2. Effects of the compounds on the viability of human breast adenocarcinoma cancer cells

The newly synthesized hydroxypyrazolines bearing 3-fluoro-4methoxyphenyl moiety **4a**–**I** as potential anticancer agents were evaluated for their *in vitro* cytotoxicity against human breast adenocarcinoma (MCF-7, MDA-MB-231) and normal monkey kidney epithelial (VERO) cell lines using the standard MTT assay. From the data reported in Table 3, revealed that the compounds **4a**–**I** were non-toxic to the normal cells along with the cancer cell lines, i.e., on MCF-7 and MDA-MB-231, except compounds 4b, 4c, 4f, 4h and 4i. Among them, compound 4i was exhibited most potent activity against MCF-7 and MDA-MB-231 cell lines with IC₅₀ values of 1.86 \pm 0.02, 2.45 \pm 0.05 μ M, comparable to the positive control Doxorubicin. Compounds **4h** and **4c** showed promising inhibitory activity (IC_{50} = 5.44 \pm 0.03 μM for MDA-MB-231, IC_{50} = 7.98 \pm 0.03 μM for MCF-7, IC_{50} = 11.39 \pm 0.04 μM for MDA-MB-231, respectively). However, compound 4c showed equal cytotoxicity on normal VERO cell line with IC50 value $25.04 \pm 0.16 \,\mu$ M. Moreover, the compounds **4f** and **4b** were found to have selective toxicity against the cancer cell line, i.e., on MCF-7 as their IC₅₀ values were found to be 21.35 \pm 0.14, 36.69 \pm 0.06 μ M, respectively. A closer look into the structure activity relationship suggests that the cytotoxic potency was highly dependent, not surprisingly, on the substitution types and patterns on the phenyl rings. The different substituents on the two phenyl rings attached at the C-3 and C-5 of the pyrazoline ring can slightly alter the cytotoxicity against the cancer cell lines tested. However, replacing the hydrogen with an electron donating group on the phenyl ring attached to the C-3 position of the pyrazoline ring resulted in a significant activity increase. The compounds with methoxy, methyl groups in **4i**, **4h** and **4c** were more effective in inhibiting cancer cell growth, which was probably caused by favorable steric interactions of the bulky hydrophobic groups with the binding site. Also, the results clearly indicate that the halogenated substitution on the phenyl ring attached to the C-5 position of the pyrazoline ring showed better activity against the MCF-7 cancer cell line. Hence, the biological response increased by halogenated analogs than their non-halogenated motifs. This is probably due to enhanced lipophilicity, pharmacokinetic properties, physicochemical properties, endurance for metabolic destruction and electronegativity.

4.3. Antioxidant activity against DPPH radical

A perusal of Table 4 reveals that all the tested compounds possessed moderate to good antioxidant activity and indicates that radical scavenging activity in DPPH increases with concentration. The maximum radical scavenging activity has been exhibited by compounds **4i**, **4h** followed closely by **4c**, since it is evident from their IC₅₀ values 16.08 μ g/mL, 16.61 μ g/mL and 17.70 μ g/mL



Fig. 2. Molecular structures of compounds (a) 4b and (b) 4c showing the atomic numbering. The displacement ellipsoids are drawn at 50% probability.

Table 2

Crystallographic data and structure refinement details for **4b** and **4c**.

	Compound 4b	Compound 4c
CCDC deposit No.	CCDC 1406922	CCDC 1406977
Empirical formula	C ₂₄ H ₁₉ Cl ₂ FN ₂ O ₃	C24H19Cl2FN2O4
Formula weight	473.31	489.31
Temperature	293(2) K	293(2) K
Wavelength	1.54178 Å	1.54178 Å
Crystal system, space group	Monoclinic, P2 ₁ /c	Monoclinic, P2 ₁ /c
Unit cell dimensions	a = 13.5196(10) Å	a = 14.7599(18) Å
	b = 12.7542(8) Å	b = 12.6135(15) Å
	c = 12.7141(9) Å	c = 12.2612(14) Å
	$eta=97.219(4)^\circ$	$eta=97.505(7)^\circ$
Volume	2174.9(3) Å ³	2263.2(5) Å ³
Z, Calculated density	4, 1.445 Mg/m ³	4, 1.436 Mg/m ³
Absorption correction	Multi-scan	Multi-scan
Absorption coefficient	3.017 mm^{-1}	2.953 mm^{-1}
$F_{(000)}$	976	1008
Crystal size	$0.3 \times 0.27 \times 0.25 \text{ mm}$	$0.28 \times 0.26 \times 0.23~mm$
Theta range for data collection	3.29°-64.61°	5.05°-64.50°
Limiting indices	$-15 \leq h \leq 15$	$-17 \le h \le 16$
	$-14 \leq k \leq 14$	$-14 \leq k \leq 13$
	$-14 \le l \le 12$	$-14 \le l \le 14$
Reflections collected/unique	$18210/3608 [R_{int} = 0.0831]$	$16502/3690 [R_{int} = 0.0993]$
Refinement method	Full-matrix least-squares on F^2	Full-matrix least-squares on F^2
Data/restraints/parameters	3608/0/291	3690/0/301
Goodness-of-fit on F ²	1.036	0.997
Final R indices $[I > 2\sigma(I)]$	R1 = 0.0639, <i>w</i> R2 = 0.1712	R1 = 0.0482, wR2 = 0.1249
R indices (all data)	R1 = 0.0858, wR2 = 0.1888	R1 = 0.0979, wR2 = 0.1635
Largest diff. peak and hole	1.105 and -0.669 e. Å ⁻³	0.348 and -0.376 e. Å $^{-3}$

Table 3

The *in vitro* cytotoxic activity of **4a–1** on cancer and normal cells by MTT assay at 48 h of exposure.

Compound	IC ₅₀ ^a in µM			
	MDA-MB-231	MCF-7	VERO	
4a	>100	>100	>100	
4b	>100	36.69 ± 0.06	>100	
4c	11.39 ± 0.04	7.98 ± 0.03	25.04 ± 0.16	
4d	>100	>100	>100	
4e	>100	>100	>100	
4f	>100	21.35 ± 0.14	>100	
4g	>100	>100	>100	
4h	5.44 ± 0.03	>100	>100	
4i	2.45 ± 0.05	1.86 ± 0.02	>100	
4j	>100	>100	>100	
4k	>100	>100	>100	
41	>100	>100	>100	
Doxorubicin ^D	0.91 ± 0.19	1.08 ± 0.02	>100	

 $^a\,$ Data presented is the mean \pm SD value of three independent determinations. $^b\,$ Positive control.

respectively, whereas the IC₅₀ value of the standard ascorbic acid in DPPH method was found to be 15.17 μ g/mL at 25 μ g/mL. It is clear from the results that the antioxidant potential of compounds is connected with the positioning and forms of substituents on the phenyl rings. In addition, the enhancement in activity due to the presence of a hydroxy group at position C-5 carbon and 3-fluoro 4methoxyphenyl group attached to C-1 carbon of the pyrazoline ring. Compounds 4i and 4h, which have electron donating methoxy and methyl substitutions, respectively, at the para position of phenyl rings attached to C-3 and C-5 carbons of the pyrazoline ring exerted excellent activity. Compound 4c, which has an electron donating methoxy group at the para position of the phenyl ring attached to C-3 and an electron withdrawing chloro substitution at the ortho-para position of the phenyl ring attached to C-5 carbons of the pyrazoline ring, contributed in the radical scavenging ability. Compounds **4I**, **4g**, **4j** and **4f** showed moderate antioxidant activity,

Table 4 The in vitro antioxidant activity of 4a–1 in DPPH method.

Compound	Concentration (µg/mL)					
	25	50	100	IC ₅₀		
4a	_	_	_	_		
4b	51.37 ± 1.23	54.05 ± 0.86	63.72 ± 0.63	24.33 ± 0.30		
4c	70.60 ± 0.25	74.21 ± 0.43	81.86 ± 0.70	17.70 ± 0.56		
4d	48.65 ± 0.60	52.86 ± 1.24	61.93 ± 0.80	25.69 ± 0.12		
4e	-	-	-	-		
4f	63.87 ± 0.30	65.49 ± 0.46	72.54 ± 0.78	19.57 ± 0.23		
4g	66.28 ± 1.16	69.53 ± 1.31	74.19 ± 0.71	18.85 ± 0.60		
4h	75.23 ± 0.18	79.54 ± 0.36	85.44 ± 0.60	16.61 ± 0.56		
4i	77.69 ± 0.24	83.26 ± 0.42	86.20 ± 0.82	16.08 ± 0.75		
4j	64.12 ± 0.22	67.44 ± 0.64	72.59 ± 0.78	19.49 ± 0.26		
4k	53.02 ± 0.14	56.61 ± 0.33	64.28 ± 0.50	23.57 ± 0.43		
41	68.42 ± 0.28	71.68 ± 0.42	79.66 ± 0.82	18.26 ± 0.18		
Ascorbic acid	82.39 ± 0.12	83.52 ± 0.38	87.22 ± 0.54	15.17 ± 0.44		
Blank	-	-	-	_		

(-) Showed no scavenging activity; Values were the means of three replicates \pm SD.

whereas the other compounds **4k**, **4b** and **4d** displayed mild activity. Structure–activity relationship (SAR) reveals that the electron-donating groups are generally more beneficial than the electron withdrawing or unsubstituted groups in the phenyl rings.

5. Conclusions

In short, we have synthesized a new, biologically active 1,3,5trisubstituted aryl-5-hydroxypyrazoline derivatives bearing 3fluoro-4-methoxyphenyl moiety and their structures were characterized by their spectral and analytical data. The newly synthesized analogues were evaluated for their *in vitro* anticancer activity against breast adenocarcinoma cell lines (MCF-7, MDA-MB-231) and *in vitro* antioxidant activity. Among the analogue compounds, **4i** demonstrated most potent activity against MCF-7 and MDA-MB-231 cell lines, while **4c** and **4h** exhibited moderate toxicity in cancer cell lines. Moreover, the compounds **4f** and **4b** were found to have selective toxicity against the breast cancer cell line, i.e., on MCF-7. *In vitro* antioxidant studies revealed that compounds **4i**, **4h** and **4c** showed excellent activity in comparison to the standard drug. The significant anticancer and antioxidant activities of the synthesized compounds may be due to the presence of the electron-donating and halo substituted groups on the phenyl rings along with core hydroxypyrazoline moiety. It is clear from the results that the cytotoxic effect of these derivatives is in conjunction with their antioxidant activities may be looked at as key steps for the designing more potent new chemical entities with comparable pharmacological profiles to that of the standard drugs.

6. Experimental protocols

6.1. Materials and methods

Melting point was taken in open capillary tube and was uncorrected. The purity of the compound was confirmed by thin layer chromatography using Merck silica gel 60 F₂₅₄ coated aluminum plates. Products were characterized by spectroscopy data (IR, ¹H NMR ¹³C NMR and LC-MS). IR spectrum was recorded on Shimadzu-FT-IR Infrared spectrometer in KBr (ν_{max} in cm⁻¹). ¹H NMR and ¹³C NMR spectra were recorded on 400 MHz and 100 MHz Bruker AMX 400 spectrometer, with 5 mm PABBO BB-1H TUBES with TMS as internal standard in DMSO/CDCl₃. LC-MS was obtained using Agilent 1200 series LC and Micromass zQ spectrometer. Elemental analysis was carried out by using VARIO EL-III (Elementar Analysensysteme GmBH). Chemicals were purchased from Sigma–Aldrich, India and used without further purification.

6.2. Procedure for the synthesis of chalcone dibromides (2a-l)

To a solution of chalcones 1a-1 (0.01 mol) in chloroform (50 mL), bromine (0.01 mol) in chloroform (25 mL) was added slowly with stirring. After the completion of addition of bromine solution, the reaction mixture was stirred for 24 h. Excess of chloroform was distilled off under reduced pressure. The precipitated solid was filtered, dried and recrystallized from chloroform.

6.3. Procedure for the synthesis of 1,3,5-trisubstituted aryl-5hydroxy pyrazolines (**4a–1**)

To a mixture of chalcone dibromides 2a-l (0.01 mol) in absolute ethanol (75 mL) 3-fluoro-4-methoxybenzohydrazide **3** (0.01 mol) and triethylamine (10 mL) were added. The reaction mixture was heated under reflux for ~12 h on a water bath. The contents were reduced, cooled and poured onto crushed ice and kept overnight. The resulting hydroxy pyrazolines 4a-l were collected by filtration and recrystallized from ethanol.

6.3.1. (5-(2,4-Dichlorophenyl)-5-hydroxy-3-phenyl-4,5-

dihydropyrazol-1-yl) (3-fluoro-4-methoxy phenyl)methanone (4a)

IR (KBr) v_{max} (cm⁻¹): 3353 (OH), 3099 (Ar C–H), 2945 (C–H), 1634 (C=O), 1570 (C=C), 1181 (C–F); ¹H NMR (400 MHz, CDCl₃, ppm): δ 3.84 (d, 1H, CH₂, *J* = 18.2 Hz), 3.90 (d, 1H, CH₂, *J* = 18.2 Hz), 3.95 (s, 3H, OCH₃), 5.53 (s, 1H, OH), 6.87 (d, 1H, *J* = 8.8 Hz), 7.35 (d, 2H, *J* = 7.5 Hz), 7.42 (d, 2H, *J* = 7.5 Hz), 7.47 (dd, 1H, *J* = 2.3 Hz), 7.49 (d, 1H, *J* = 2.3 Hz), 7.82–7.98 (m, 3H), 8.13 (d, 1H, *J* = 5.8 Hz); ¹³C NMR (100 MHz, DMSO-*d*₆, ppm): δ 48.4 (C-4), 55.9 (OCH₃), 91.4 (C-5), 113.3 (C-5'), 117.5 (d, ²*J*_{C–F} = 20 Hz, C-2'), 124.1, 127.2 (d, ³*J*_{C–F} = 7 Hz, C-1'), 127.8 (C-6'), 128.1, 128.4, 129.3, 130.2, 131.7, 133.6, 134.2, 134.8, 140.2, 149.8 (d, ²*J*_{C–F} = 12 Hz, C-4'), 152.4 (d, ¹*J*_{C–F} = 240 Hz, C-3'), 153.1, 163.4 (C=O); LC-MS (*m*/*z*, %): 457 ([M–H]⁻, 99), 459 (([M–H]+2)⁻, 67), 461 (([M–H]+4)⁻, 17).

6.3.2. (5-(2,4-Dichlorophenyl)-5-hydroxy-3-p-tolyl-4,5-

dihydropyrazol-1-yl) (3-fluoro-4-methoxy phenyl)methanone (**4b**) IR (KBr) v_{max} (cm⁻¹): 3361 (OH), 3083 (Ar C–H), 2926 (C–H), 1625 (C=O), 1574 (C=C), 1170 (C–F); ¹H NMR (400 MHz, CDCl₃, ppm): δ 2.27 (s, 3H, CH₃), 3.68 (d, 1H, CH₂, *J* = 18 Hz), 3.80 (d, 1H, CH₂, *J* = 18 Hz), 3.94 (s, 3H, OCH₃), 5.48 (s, 1H, OH), 6.98 (d, 1H, *J* = 8.3 Hz), 7.03–7.29 (m, 4H), 7.40 (dd, 1H, *J* = 2.4 Hz), 7.43 (d, 1H, *J* = 2 Hz), 7.76–7.89 (m, 3H); ¹³C NMR (100 MHz, DMSO-*d*₆, ppm): δ 21.6 (CH₃), 48.6 (C-4), 56.8 (OCH₃), 92.6 (C-5), 113.5 (C-5'), 116.6 (d, ²*J*_{C-F} = 18 Hz, C-2'), 123.8, 127.6 (d, ³*J*_{C-F} = 7 Hz, C-1'), 128.0 (C-6'), 128.6, 129.4, 130.2, 130.7, 131.3, 132.5, 134.2, 140.2, 142.3, 149.5 (d, ²*J*_{C-F} = 11 Hz, C-4'), 152.0 (d, ¹*J*_{C-F} = 240 Hz, C-3'), 154.5, 165.5 (C= O); LC-MS (*m*/*z*, %): 471 ([M–H]⁻, 98), 473 (([M–H]+2)⁻, 68), 475 (([M–H]+4)⁻, 13).

6.3.3. (5-(2,4-Dichlorophenyl)-5-hydroxy-3-(4-methoxyphenyl)-4,5-dihydropyrazol-1-yl) (3-fluoro-4-methoxyphenyl)methanone (4c)

IR (KBr) v_{max} (cm⁻¹): 3373 (OH), 3097 (Ar C–H), 2935 (C–H), 1614 (C=O), 1566 (C=C), 1176 (C–F); ¹H NMR (400 MHz, CDCl₃, ppm): δ 3.57 (d, 1H, CH₂, *J* = 18 Hz), 3.66 (d, 1H, CH₂, *J* = 18 Hz), 3.86 (s, 3H, OCH₃), 3.96 (s, 3H, OCH₃), 5.44 (s, 1H, OH), 6.97 (d, 2H, *J* = 8.8 Hz), 7.02 (d, 1H, *J* = 8.4 Hz), 7.37 (dd, 1H, *J* = 2.4 Hz), 7.39 (d, 1H, *J* = 2 Hz), 7.69 (d, 2H, *J* = 8.8 Hz), 7.85–7.94 (m, 3H); ¹³C NMR (100 MHz, DMSO-*d*₆, ppm): δ 48.9 (C-4), 55.8 (OCH₃), 56.6 (OCH₃), 91.1 (C-5), 113.1 (C-5'), 114.8, 117.8 (d, ²*J*_{C-F} = 19 Hz, C-2'), 123.9, 127.2 (d, ³*J*_{C-F} = 6 Hz, C-1'), 127.3, 127.7 (C-6'), 128.6, 130.1, 130.6, 131.2, 133.2, 139.2, 149.9 (d, ²*J*_{C-F} = 11 Hz, C-4'), 151.8 (d, ¹*J*_{C-F} = 242 Hz, C-3'), 153.2, 161.5, 162.8 (C=O); LC-MS (*m/z*, %): 487 ([M–H]⁻, 99), 489 (([M–H]+2)⁻, 69), 491 (([M–H]+4)⁻, 14).

6.3.4. (5-(2,4-Dichlorophenyl)-3-(4-fluorophenyl)-5-hydroxy-4,5dihydropyrazol-1-yl) (3-fluoro-4-methoxyphenyl)methanone (4d)

IR (KBr) v_{max} (cm⁻¹): 3314 (OH), 3099 (Ar C–H), 2937 (C–H), 1606 (C=O), 1555 (C=C), 1208 (C–F); ¹H NMR (400 MHz, CDCl₃, ppm): δ 3.75 (d, 1H, CH₂, *J* = 18.2 Hz), 3.83 (d, 1H, CH₂, *J* = 18.2 Hz), 3.91 (s, 3H, OCH₃), 5.56 (s, 1H, OH), 6.79 (d, 1H, *J* = 8.8 Hz), 7.12–7.25 (m, 4H), 7.42 (dd, 1H, *J* = 2.3 Hz), 7.46 (d, 1H, *J* = 2.3 Hz), 7.78–7.92 (m, 3H); ¹³C NMR (100 MHz, DMSO-*d*₆, ppm): δ 47.9 (C-4), 56.7 (OCH₃), 91.8 (C-5), 112.6 (C-5'), 116.2 (d, ²*J*_{C–F} = 21 Hz, C-2'), 117.1 (d, ²*J*_{C–F} = 22 Hz), 117.4 (d, ²*J*_{C–F} = 22 Hz), 124.3, 127.3 (d, ³*J*_{C–F} = 7 Hz, C-1'), 127.6 (C-6'), 129.4, 130.1, 130.4 (d, ⁴*J*_{C–F} = 2 Hz), 130.9 (d, ³*J*_{C–F} = 10 Hz), 131.2 (d, ³*J*_{C–F} = 10 Hz), 131.7, 134.0, 140.0, 149.7 (d, ²*J*_{C–F} = 254 Hz), 163.3 (C=O); LC-MS (*m*/*z*, %): 475 ([M–H]⁻, 85), 477 (([M–H]+2)⁻, 67), 479 (([M–H]+4)⁻, 18).

6.3.5. (3-(3-Chloro-2-fluorophenyl)-5-(2,4-dichlorophenyl)-5hydroxy-4,5-dihydropyrazol-1-yl) (3-fluoro-4-methoxyphenyl) methanone (**4e**)

IR (KBr) v_{max} (cm⁻¹): 3362 (OH), 3085 (Ar C–H), 2922 (C–H), 1627 (C=O), 1580 (C=C), 1185 (C–F); ¹H NMR (400 MHz, CDCl₃, ppm): 3.92 (d, 1H, CH₂, *J* = 18 Hz), 4.01 (d, 1H, CH₂, *J* = 18 Hz), 4.33 (s, 3H, OCH₃), 5.75 (s, 1H, OH), 7.35 (d, 1H, *J* = 8.4 Hz), 7.57 (dd, 1H, *J* = 2 Hz), 7.63 (d, 1H, *J* = 2 Hz), 7.74–7.90 (m, 3H), 8.06–8.27 (m, 3H); ¹³C NMR (100 MHz, DMSO-*d*₆, ppm): δ 48.6 (C-4), 56.2 (OCH₃), 91.8 (C-5), 113.6 (C-5'), 116.2 (d, ²*J*_{C–F} = 20 Hz, C-2'), 119.6 (d, ²*J*_{C–F} = 19 Hz), 121.2 (d, ²*J*_{C–F} = 18 Hz), 123.5, 126.4, 127.2 (d, ³*J*_{C–F} = 8 Hz, C-1'), 127.5 (C-6'), 127.8 (d, ³*J*_{C–F} = 7 Hz), 130.3, 130.9, 131.6, 132.3 (d, ³*J*_{C–F} = 9 Hz), 133.8, 139.1, 149.4 (d, ²*J*_{C–F} = 12 Hz, C-4'), 151.6 (d, ¹*J*_{C–F} = 243 Hz, C-3'), 153.2, 162.8 (d, ¹*J*_{C–F} = 248 Hz), 163.7 (C=O); LC-MS (*m*/*z*, %): 509 ([M–H]⁻, 97), 511 (([M–H]+2)⁻, 95), 513 (([M–H]+4)⁻, 32).

6.3.6. (5-(2,4-Dichlorophenyl)-3-(3-fluoro-4-methylphenyl)-5hydroxy-4,5-dihydropyrazol-1-yl) (3-fluoro-4-methoxyphenyl) methanone **(4f)**

IR (KBr) v_{max} (cm⁻¹): 3387 (OH), 3097 (Ar C–H), 2935 (C–H), 1620 (C=O), 1566 (C=C), 1178 (C–F); ¹H NMR (400 MHz, CDCl₃, ppm): δ 2.76 (s, 3H, CH₃), 3.97 (d, 1H, CH₂, *J* = 18 Hz), 4.08 (d, 1H, CH₂, *J* = 18 Hz), 4.40 (s, 3H, OCH₃), 5.86 (s, 1H, OH), 7.44 (d, 1H, *J* = 8.4 Hz), 7.65 (dd, 1H, *J* = 2 Hz), 7.78 (d, 1H, *J* = 2 Hz), 7.80–7.85 (m, 3H), 8.23–8.37 (m, 3H); ¹³C NMR (100 MHz, DMSO-*d*₆, ppm): δ 14.7 (CH₃), 48.8 (C-4), 56.6 (OCH₃), 91.3 (C-5), 113.0 (C-5'), 113.4 (d, ²*J*_{C-F} = 17 Hz), 117.8 (d, ²*J*_{C-F} = 20 Hz, C-2'), 122.9, 124.1, 126.9 (d, ²*J*_{C-F} = 16 Hz), 127.2, 127.4 (d, ³*J*_{C-F} = 8 Hz, C-1'), 127.7 (C-6'), 130.1, 130.6, 131.3 (d, ³*J*_{C-F} = 8 Hz), 132.6 (d, ³*J*_{C-F} = 7 Hz), 133.3, 139.0, 150.0 (d, ²*J*_{C-F} = 10 Hz, C-4'), 151.9 (d, ¹*J*_{C-F} = 242 Hz, C-3'), 152.6, 162.4 (d, ¹*J*_{C-F} = 242 Hz), 163.1 (C=O); LC-MS (*m*/*z*, %): 489 ([M–H]⁻, 98), 491 (([M–H]+2)⁻, 58), 493 (([M–H]+4)⁻, 10).

6.3.7. (3-Fluoro-4-methoxyphenyl) (5-hydroxy-3-phenyl-5-p-tolyl-4,5-dihydropyrazol-1-yl)methanone (**4g**)

IR (KBr) v_{max} (cm⁻¹): 3393 (OH), 3062 (Ar C–H), 2924 (C–H), 1641 (C=O), 1583 (C=C), 1181 (C–F); ¹H NMR (400 MHz, DMSO-*d*₆, ppm): δ 2.29 (s, 3H, CH₃), 3.40 (d, 1H, CH₂, *J* = 18.2 Hz), 3.53 (d, 1H, CH₂, *J* = 18.2 Hz), 3.95 (s, 3H, OCH₃), 6.92 (s, 1H, OH), 7.18 (d, 2H, *J* = 8 Hz), 7.26 (d, 2H, *J* = 7.3 Hz), 7.34 (d, 2H, *J* = 7.3 Hz), 7.59 (d, 2H, *J* = 8 Hz), 7.73–7.81 (m, 3H), 7.96 (d, 1H, *J* = 5.9 Hz); ¹³C NMR (100 MHz, DMSO-*d*₆, ppm): δ 21.8 (CH₃), 51.1 (C-4), 56.4 (OCH₃), 93.8 (C-5), 112.9 (C-5'), 117.6 (d, ²*J*_{C–F} = 21 Hz, C-2'), 125.2, 126.5, 127.4 (d, ³*J*_{C–F} = 6 Hz, C-1'), 127.7 (C-6'), 129.0, 129.8, 134.8, 135.1, 136.9, 140.5, 149.8 (d, ²*J*_{C–F} = 10 Hz, C-4'), 151.5 (d, ¹*J*_{C–F} = 244 Hz, C-3'), 153.6, 163.5 (C=O); LC-MS (*m*/*z*, %): 403 ([M–H]⁻, 95).

6.3.8. (3-Fluoro-4-methoxyphenyl) (5-hydroxy-3,5-di-p-tolyl-4,5-dihydropyrazol-1-yl)methanone **(4h)**

IR (KBr) v_{max} (cm⁻¹): 3402 (OH), 3020 (Ar C–H), 2927 (C–H), 1633 (C=O), 1577 (C=C), 1176 (C–F); ¹H NMR (400 MHz, DMSO- d_6 , ppm): δ 2.27 (s, 3H, CH₃), 2.33 (s, 3H, CH₃), 3.44 (d, 1H, CH₂, J = 18.4 Hz), 3.59 (d, 1H, CH₂, J = 18.4 Hz), 3.91 (s, 3H, OCH₃), 6.90 (s, 1H, OH), 7.15 (d, 2H, J = 8 Hz), 7.24–7.27 (m, 3H), 7.38 (d, 2H, J = 8 Hz), 7.61 (d, 2H, J = 8 Hz), 7.69–7.76 (m, 2H); ¹³C NMR (100 MHz, DMSO- d_6 , ppm): δ 21.0 (CH₃), 21.5 (CH₃), 51.5 (C-4), 56.6 (OCH₃), 94.1 (C-5), 113.1 (C-5'), 117.8 (d, ² J_{C-F} = 19 Hz, C-2'), 125.0, 126.9, 127.5 (C-6'), 127.7 (d, ³ J_{C-F} = 6 Hz, C-1'), 128.9, 129.0, 129.8, 136.7, 140.6, 141.4, 149.9 (d, ² J_{C-F} = 11 Hz, C-4'), 151.9 (d, ¹ J_{C-F} = 242 Hz, C-3'), 153.0, 164.0 (C=O); LC-MS (m/z, %): 417 ([M–H]⁻, 97).

6.3.9. (3-Fluoro-4-methoxyphenyl) (5-hydroxy-3-(4-

methoxyphenyl)-5-p-tolyl-4,5-dihydropyr azol-1-yl)methanone **(4i)** IR (KBr) ν_{max} (cm⁻¹): 3425 (OH), 3033 (Ar C–H), 2930 (C–H), 1621 (C=O), 1565 (C=C), 1184 (C–F); ¹H NMR (400 MHz, DMSO- d_6 , ppm): δ 2.29 (s, 3H, CH₃), 3.49 (d, 1H, CH₂, *J* = 18 Hz), 3.63 (d, 1H, CH₂, *J* = 18 Hz), 3.83 (s, 3H, OCH₃), 3.96 (s, 3H, OCH₃), 6.89 (d, 2H, *J* = 8.5 Hz), 6.97 (s, 1H, OH), 7.02 (d, 2H, *J* = 8.1 Hz), 7.20 (d, 2H, *J* = 8.5 Hz), 7.41 (d, 2H, *J* = 8.1 Hz), 7.81–7.93 (m, 3H); ¹³C NMR (100 MHz, DMSO- d_6 , ppm): δ 21.0 (CH₃), 51.9 (C-4), 55.7 (OCH₃), 56.2 (OCH₃), 94.3 (C-5), 113.6 (C-5'), 114.7, 116.8 (d, ²*J*_{C–F} = 19 Hz, C-2'), 125.2, 127.1, 127.5 (d, ³*J*_{C–F} = 7 Hz, C-1'), 127.7, 128.2 (C-6'), 129.5, 136.7, 139.5, 149.6 (d, ²*J*_{C–F} = 12 Hz, C-4'), 152.0 (d, ¹*J*_{C–F} = 240 Hz, C-3'), 153.8, 161.3, 164.2 (C=O); LC-MS (*m*/*z*, %): 433 ([M–H]⁻, 99).

6.3.10. (3-Fluoro-4-methoxyphenyl) (3-(4-fluorophenyl)-5hydroxy-5-p-tolyl-4,5-dihydropyr azol-1-yl)methanone (4j)

IR (KBr) v_{max} (cm⁻¹): 3414 (OH), 3095 (Ar C–H), 2918 (C–H), 1620 (C=O), 1512 (C=C), 1165 (C–F); ¹H NMR (400 MHz, DMSO-*d*₆, ppm): δ 2.28 (s, 3H, CH₃), 3.47 (d, 1H, CH₂, *J* = 18.4 Hz), 3.61 (d, 1H,

CH₂, *J* = 18.4 Hz), 3.91 (s, 3H, OCH₃), 6.95 (s, 1H, OH), 7.15 (d, 2H, *J* = 8.4 Hz), 7.23–7.32 (m, 3H) 7.39 (d, 2H, *J* = 8.4 Hz), 7.68–7.78 (m, 4H); ¹³C NMR (100 MHz, DMSO-*d*₆, ppm): δ 22.1 (CH₃), 53.5 (C-4), 56.6 (OCH₃), 94.7 (C-5), 112.8 (C-5'), 114.9 (d, ²*J*_{C-F} = 21 Hz, C-2'), 116.2 (d, ²*J*_{C-F} = 20 Hz), 116.7 (d, ²*J*_{C-F} = 20 Hz), 124.6, 126.8, 127.2 (C-6'), 127.6 (d, ³*J*_{C-F} = 7 Hz, C-1'), 129.5 (d, ⁴*J*_{C-F} = 1.6 Hz), 129.9 (d, ³*J*_{C-F} = 11 Hz), 130.3 (d, ³*J*_{C-F} = 11 Hz), 136.5, 140.7, 150.1 (d, ²*J*_{C-F} = 25 Hz), 164.9 (C=O); LC-MS (*m*/*z*, %): 421 ([M–H]⁻, 97).

6.3.11. (3-(3-Chloro-2-fluorophenyl)-5-hydroxy-5-p-tolyl-4,5-

dihydropyrazol-1-yl) (3-fluoro-4-meth oxyphenyl)methanone **(4k)** IR (KBr) v_{max} (cm⁻¹): 3397 (OH), 3085 (Ar C–H), 2930 (C–H), 1625 (C=O), 1536 (C=C), 1178 (C–F); ¹H NMR (400 MHz, DMSO- d_6 , ppm): δ 2.30 (s, 3H, CH₃), 3.50 (d, 1H, CH₂, *J* = 18.2 Hz), 3.65 (d, 1H, CH₂, *J* = 18.2 Hz), 3.98 (s, 3H, OCH₃), 6.97 (s, 1H, OH), 7.32 (d, 2H, *J* = 8.4 Hz), 7.54 (d, 2H, *J* = 8.4 Hz), 7.68–7.82 (m, 3H), 7.87–7.96 (m, 3H); ¹³C NMR (100 MHz, DMSO- d_6 , ppm): δ 22.5 (CH₃), 54.7 (C-4), 56.4 (OCH₃), 94.4 (C-5), 112.9 (C-5'), 116.4 (d, ²*J*_{C-F} = 20 Hz, C-2'), 121.2 (d, ²*J*_{C-F} = 18 Hz), 122.3 (d, ²*J*_{C-F} = 16 Hz), 124.1, 124.9, 126.7, 127.0 (d, ³*J*_{C-F} = 7 Hz, C-1'), 127.2 (C-6'), 128.3 (d, ³*J*_{C-F} = 7 Hz), 133.9 (d, ³*J*_{C-F} = 245 Hz, C-3'), 153.0, 162.8 (d, ¹*J*_{C-F} = 244 Hz), 164.7 (C=O); LC-MS (*m/z*, %): 455 ([M–H]⁻, 90).

6.3.12. (3-Fluoro-4-methoxyphenyl) (3-(3-fluoro-4-methylphenyl)-5-hydroxy-5-p-tolyl-4,5-dihydro pyrazol-1-yl)methanone **(41)**

IR (KBr) v_{max} (cm⁻¹): 3418 (OH), 3093 (Ar C–H), 2915 (C–H), 1619 (C=O), 1563 (C=C), 1165 (C–F); ¹H NMR (400 MHz, DMSO-*d*₆, ppm): δ 2.28 (s, 3H, CH₃), 2.36 (s, 3H, CH₃), 3.48 (d, 1H, CH₂, *J* = 18.4 Hz), 3.62 (d, 1H, CH₂, *J* = 18.4 Hz), 3.97 (s, 3H, OCH₃), 6.96 (s, 1H, OH), 7.34 (d, 2H, *J* = 8 Hz), 7.58 (d, 2H, *J* = 8 Hz), 7.83–7.92 (m, 3H), 8.13–8.27 (m, 3H); ¹³C NMR (100 MHz, DMSO-*d*₆, ppm): δ 14.9 (CH₃), 21.3 (CH₃), 49.6 (C-4), 56.3 (OCH₃), 94.0 (C-5), 112.8 (C-5'), 113.0 (d, ²*J*_{C-F} = 18 Hz), 116.1 (d, ²*J*_{C-F} = 20 Hz, C-2'), 124.0, 125.6, 126.3 (d, ²*J*_{C-F} = 16 Hz), 127.2, 127.5 (d, ³*J*_{C-F} = 9 Hz, C-1'), 128.0 (C-6') 131.1 (d, ³*J*_{C-F} = 8 Hz), 132.8 (d, ³*J*_{C-F} = 8 Hz), 136.1, 140.4, 150.2 (d, ²*J*_{C-F} = 11 Hz, C-4'), 151.7 (d, ¹*J*_{C-F} = 238 Hz, C-3'), 154.5, 163.4 (d, ¹*J*_{C-F} = 246 Hz), 165.1 (C=O); LC-MS (*m*/*z*, %): 435 ([M–H]⁻, 98).

Acknowledgments

The authors are thankful to The Head, NMR Centre, IISc Bangalore and UGC-BSR for the financial assistance provided to one of the authors, Dinesha. The authors also grateful to the Institution of Excellence, Vijnana Bhavana, University of Mysore, for providing the single-crystal X-ray diffractometer facility.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2015.09.029.

References

- (a) A. Jemal, F. Bray, M.M. Center, J. Ferlay, E. Ward, D. Forman, CA Cancer J. Clin. 61 (2011) 69–90;
 (b) B.W. Stewart, C.P. Wild, World Cancer Report-2014, International Agency
- for Research on Cancer, Lyon, France, 2014. [2] G.A. Elmegeed, W.K.B. Khalil, R.M. Mohareb, H.H. Ahmed, M.M. Abd-Elhalim,
- G.H. Elsayed, Bioorg. Med. Chem. 19 (2011) 6860–6872.
 [3] M.I. Ansari, M.K. Hussain, A. Arun, B. Chakravarti, R. Konwar, K. Hajela, Eur. J.
- Med. Chem. 99 (2015) 113–124. [4] M. Zhou, S. Ji, Z. Wu, Y. Li, W. Zheng, H. Zhou, T. Chen, Eur. J. Med. Chem. 96
- (2015) 92–97.
 [5] N. Magne, C. Chargari, D. MacDermed, R. Conforti, L. Vedrine, J.P. Spano,
- [5] N. Magne, C. Chargari, D. MacDernieu, K. Conorti, L. Vennie, J.P. Span D. Khayat, Crit. Rev. Oncol. Hematol. 76 (2010) 186–195.
 [6] V.C. Jorden, W.J. Gradishar, Mol. Asp. Med. 18 (1997) 187–247.

31

- [7] M. Baum, A.U. Budzar, J. Cuzick, J. Forbes, J.H. Houghton, J.G. Klijn, T. Sahmoud, Lancet 359 (2002) 2131-2139.
- [8] J. Rangaswamy, H. Vijay Kumar, S.T. Harini, N. Naik, Bioorg. Med. Chem. Lett. 22 (2012) 4773-4777.
- [9] S. Emami, S. Dadashpour, Eur. J. Med. Chem. 102 (2015) 611-630.
- [10] V.C. Jorden, J. Med. Chem. 46 (2003) 1081–1111.
- [11] Y.R. Huang, J.A. Katzenellenbogen, Org. Lett. 2 (2000) 2833–2836.
- [12] V.R. Tandon, S. Sharma, A. Mahajan, G.H. Bardi, JK Sci. 7 (2005) 1–3.
- [13] Dinesha, S. Viveka, P. Naik, G.K. Nagaraja, Med. Chem. Res. 23 (2014) 4189-4197.
- [14] S. Viveka, Dinesha, L.N. Madhu, G.K. Nagaraja, Monatsh. Chem. 146 (2015) 1547-1555.
- [15] T. Taj, R.R. Kamble, T.M. Gireesh, R.K. Hunnur, S.B. Margankop, Eur. J. Med. Chem. 46 (2011) 4366-4373.
- [16] A.M. Pisoschi, A. Pop, Eur, J. Med. Chem. 97 (2015) 55–74.
 [17] F.M. Awadallah, G.A. Piazza, B.D. Gary, A.B. Keeton, J.C. Canzoneri, Eur. J. Med. Chem 70 (2013) 273–279
- [18] D. Havrylyuk, B. Zimenkovsky, O. Vasylenko, C.W. Day, D.F. Smee, P. Grellier, R. Lesyk, Eur. J. Med. Chem. 66 (2013) 228–237.
- [19] S.L. Zhu, Y. Wu, C.J. Liu, C.Y. Wei, J.C. Tao, H.M. Liu, Eur. J. Med. Chem. 65 (2013) 70-82
- [20] T.S. Jeong, K.S. Kim, J.R. Kim, K.H. Cho, S. Lee, W.S. Lee, Bioorg. Med. Chem. Lett. 14 (2004) 2719-2723.
- [21] B. Insuasty, A. Montoya, D. Becerra, J. Quiroga, R. Abonia, S. Robledo, I. Darío Vélez, Y. Upegui, M. Nogueras, J. Cobo, Eur. J. Med. Chem. 67 (2013) 252–262.
- [22] R. Aggarwal, A. Bansal, I. Rozas, B. Kelly, P. Kaushik, D. Kaushik, Eur. J. Med. Chem. 70 (2013) 350-357.
- [23] D. Havrylyuk, B. Zimenkovsky, O. Karpenko, P. Grellier, R. Lesyk, Eur. J. Med. Chem. 85 (2014) 245-254.
- [24] N. Gokhan-Kelekci, S. Yabanoglu, E. Kupeli, U. Salgin, O. Ozgen, G. Ucar, E. Yesilada, E. Kendi, A. Yesilade, A.A. Bilgin, Bioorg. Med. Chem. 15 (2007) 5775-5786.
- [25] J.H.M. Lange, H.K.A.C. Coolen, H.H. Van Stuivenberg, J.A.R. Dijksman,

A.H.J. Herremans, E. Ronken, H.G. Keizer, K. Tipker, A.C. McCreary, W. Veerman, H.C. Wals, B. Stork, P.C. Verveer, A.P. Den Hartog, N.M.J. De Jong, T.J.P. Adolfs, J. Hoogendoorn, C.G. Kruse, J. Med. Chem. 47 (2004) 627-643.

- [26] Y.R. Prasad, A.L. Rao, K. Prasoona, K. Murali, P.R. Kumar, Bioorg. Med. Chem. Lett. 15 (2005) 5030-5034.
- [27] M.S. Karthikeyan, B.S. Holla, N.S. Kumari, Eur. J. Med. Chem. 42 (2007) 30-36.
- [28] Y. Rajendra Prasad, G.V.S. Kumar, S.M. Chandrashekar, Med. Chem. Res. 22 (2013) 2061-2078.
- [29] A. Sharma, T. Pathan, R. Mohan, S.C. Ramaa, Lett. Drug. Des. Discov. 8 (2011) 843-849.
- [30] (a) S. Samshuddin, B. Narayana, B.K. Sarojini, R. Srinivasan, Vinayachandra, K.R. Chandrashekar, Der Pharma Chem. 4 (2012) 587–592; (b) B.S. Rao, P.M. Akberali, B.S. Holla, B.K. Sarojni, J. Pharmacol. Toxicol. 3 (2008) 102–110.
- [31] Dinesha, S. Viveka, S. Chandra, G.K. Nagaraja, Monatsh. Chem. 146 (2015) 207-214.
- [32] T. Mosmann, J. Immunol. Methods 65 (1983) 55–63.
 [33] I. Dhamija, N. Kumar, S.N. Manjula, V. Parihar, M.M. Setty, K.S.R. Pai, Exp. Toxicol. Pathol. 65 (2013) 235–242.
- [34] M. Burits, F. Bucar, Phytother. Res. 14 (2000) 323–328.
- [35] M. Cuendet, K. Hostettmann, O. Potterat, W. Dyatmiko, Helv. Chim. Acta 80 (1997) 1144-1152.
- [36] I.I. Koleva, T.A. van Beek, J.P.H. Linssen, A.D. Groot, L.N. Evstatieva, Phytochem. Anal. 13 (2002) 8-17.
- [37] Bruker, APEX2, SAINT PLUS, Bruker AXS Inc., Madison, Wisconsin, U.S.A., 2009. [38] G.M. Sheldrick, A short history of SHELX, Acta Crystallogr. Sect. A 64 (2008) 112-122
- [39] A.L. Spek, PLATON, an integrated tool for the analysis of the results of a single crystal structure determination, Acta Crystallogr. Sect. A 46 (1990) C34.
- [40] C.F. Macrae, I.J. Bruno, J.A. Chisholm, P.R. Edgington, P. McCabe, E. Pidcock, L. Rodriguez-Monge, R. Taylor, J. van de Streek, P.A. Wood, J. Appl. Cryst. 41 (2008) 466-470.